PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION See Form PCT/IPEA/416	
P07087PC/ALi		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/SE2005/000413	22-03-2005	24-03-2004
International Patent Classification (IPC) or national classification and IPC		
See Supplemental Box		
Applicant		
Biochromix AB et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 5 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))
      _____, containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
23-01-2006	21-03-2006
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. +46 8 667 72 88	Authorized officer Malin Söderman/MP Telephone No. +46 8 782 25 00

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Cover sheet

International patent classification (IPC)

G01N33/53 (2006.01)
C12Q1/68 (2006.01)
G03F 7/00 (2006.01)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/SE2005/000413

Box No. I Basis of the report

1. With regard to the **language**, this report is based on:

☒ the international application in the language in which it was filed

☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of:

- ☐ international search (Rules 12.3(a) and 23.1(b))
- ☐ publication of the international application (Rule 12.4(a))
- ☐ international preliminary examination (Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

☐ the international application as originally filed/furnished

☒ the description:

pages 1-26 as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☒ the claims:

pages _____ as originally filed/furnished
pages* _____ as amended (together with any statement) under Article 19
pages* 1-5 received by this Authority on 23-01-2006
pages* _____ received by this Authority on _____

☒ the drawings:

pages 1-11 as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☐ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages _____
- ☐ the claims, Nos. _____
- ☐ the drawings, sheets/figs _____
- ☐ the sequence listing *(specify)*: _____
- ☐ any table(s) related to the sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

- ☐ the description, pages _____
- ☐ the claims, Nos. _____
- ☐ the drawings, sheets/figs _____
- ☐ the sequence listing *(specify)*: _____
- ☐ any table(s) related to the sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/SE2005/000413

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-24	YES
	Claims		NO
Inventive step (IS)	Claims	1-24	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-24	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

Reference is made to the following documents:
D1: WO20040006291 A2
D2: WO03096016 A1
D3: TEN, J.L., ET AL. Microcontact printing of proteins on mixed self-assembled monolayers. Langmuir. 2002, Vol. 18
D4: INGANÄS O, ET AL. Chip and solution detection of DNA hybridization using a luminescent zwitterionic polythiophene derivative. Nature materials. 2003, Vol. 2, No. 6

The invention relates to a patterned substrate for biosensing applications, wherein the pattern comprises hydrophilic and hydrophobic areas, and wherein a selected one of said areas comprises at least one reporter molecule, a property of which is detectable. It also relates to a method of making a patterned substrate comprising performing a stamping procedure to provide a pattern of hydrophilic and hydrophobic areas on a substrate of a suitable material. One step of the stamping procedure comprises attaching at least one reporter molecule to the selected one of said areas, the fluorescence of said conjugated polyelectrolyte being detectable and changing as a result of interaction with a biomolecule.
The reporter molecule could comprise conjugated polyelectrolytes, copolymers or homopolymers of thiophene, pyrrole, aniline, furan, phenylene or vinylene.

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

D4 describes a chip detection method of DNA hybridization using a luminescent zwitterionic polythiophene derivative, see abstract. D4 uses PDMS stamps and POWT, see page 422, right-hand column, lines 30-52. POWT can form a complex with single stranded DNA and this is detected as a decrease in the intensity and a red-shift in the fluorescence, see fig. 2, abstract. A complementary DNA-strand can bind to the ssDNA/polymer complex and this is detected as an increase in the intensity and a blue-shift in the fluorescence, see fig.2, abstract.

The cited documents represent the general state of the art.

The invention defined in claims 1-24 is not disclosed by any of these documents or a combination of the documents.

The cited prior art does not give any indication that would lead a person skilled in the art to the claimed method of making a device usable for detection of biomolecular interactions, where a pattern of hydrophilic and hydrophobic areas is formed on a substrate. Therefore, the claimed invention is not obvious to a person skilled in the art.

Accordingly, the invention defined in claims 1-24 is novel and is considered to involve an inventive step. The invention is industrially applicable.

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

D1 describes a method for patterning, see abstract. Document D1 is considered to represent the closest prior art. In D1, a PDMS stamp (hydrophobic) modifies surface energy of surfaces consisting of PEDOT/PSS and Na-PSS, see page 31, lines 1-18, claim 1. The stamp gives the PEDOT/PSS surface low surface energy and the Na-PSS surface high surface energy. It is considered that a surface that is hydrophobic has a low surface energy and a surface that is hydrophilic has a high surface energy. Hence, the surfaces described in D1 have hydrophilic and hydrophobic areas after the pattern method. The device layer that is patterned could be a polymer, see claim 3. A conjugated polymer could give photoluminescence emission which is detectable, see D1 page 31, lines 27-28.

D2 describes a complex between a conjugated polyelectrolyte and one or more receptor molecules specific for a target biomolecule analyte, see abstract. D2 also relates to a method of determining selected properties of biomolecules. D2 involves a method where the complex is exposed to a target biomolecule analyte whereby the analyte and the receptor interact, and a change of a property of said polyelectrolyte in response to the interaction between the receptor and analyte is detected. The detected change is used to determine the selected property of the biomolecule. D2 describes zwitterionic conjugated polymers that could comprise homopolymers and copolymers of thiophene, homopolymers, pyrrole, aniline, furan, phenylene and vinylene, see page 7, line 20 - page 8, line 2. The receptor molecule described in D2 could be peptides, nucleic acids, lipids, or pharmaceuticals, see page 8, lines 14-17.

D3 describes the use of mixed SAM:s on gold. D3 investigates how hydrophilic content of a substrate influences microcontact printing of fluorescently labelled proteins, see abstract, page 520, fig.1.

CLAIMS:

1. A method of making a device usable for the detection of biomolecular interactions, comprising

providing a substrate of a suitable material;

performing a stamping procedure using soft lithography to provide a pattern of hydrophilic and hydrophobic areas on said substrate;

applying an aqueous solution of at least one reporter molecule to at least selected ones of said areas, a property of said reporter molecule being detectable and capable of changing as a result of interaction with a biomolecule;

incubating the substrate with applied solution for a predetermined time;

removing excess solution; and

drying the substrate.

2. The method as claimed in claim 1, wherein said reporter molecule is selected from the group consistng of conjugated polyelectrolytes, copolymers or homopolymers of thiophene, pyrrole, aniline, furan, phenylene, vinylene or derivatives thereof.

3. The method as claimed in claim 2, wherein said conjugated polyelectrolyte is fluorescent.

4. The method as claimed in any of claims 1 - 3, wherein said reporter molecule is capable of interaction with a biomolecule, and wherein said interaction will cause a change in said detectable property.

5. The method as claimed in any of claims 1 – 4, wherein said substrate comprises silicon wafers, glass, glass slides, glass beads, glass wafers, silicon rubber, polystyrene, polyethylene, fluorinated hydrocarbon polymers, silica gel beads, gold, indium tin oxide-coated materials, filter paper made from nylon, cellulose or nitrocellulose, standard copy paper or variants thereof and separation media or other chromatographic media

6. The method as claimed in any of claims 1 – 5, wherein said stamping procedure further comprising attaching to selected ones of said areas any of one

or more receptor molecules and one or more target analytes alone or in combination, and forming a complex with said reporter molecule.

7. The method as claimed in claim 6, wherein said receptor molecules are selected from the group consisting of peptides, carbohydrates, nucleic acids, lipids, pharmaceuticals, antigens, antibodies, proteins, organic polymers or combination of these molecules capable of interacting with said target analyte.

8. The method as claimed in any of claims 6 or 7, wherein said target analytes are selected from the group consisting of cells, viruses, bacteria, spores, microorganisms, peptides, carbohydrates, nucleic acids, lipids, pharmaceuticals, antigens, antibodies, proteins, enzymes, toxins, organic polymers or combinations of these molecules that are capable of interacting with said receptors or reporter/receptor complexes.

9. The method as claimed in any of claims 1 - 8, wherein the stamping procedure comprises the following steps:

bringing a patterned or non-patterned stamp into conformal contact with the substrate for a period of time, the stamp being capable of modifying the surface of the substrate to exhibit said hydrophilic and hydrophobic areas;

placing a solution containing one or more of a reporter molecule, a target analyte, a receptor molecule or a complex between two or more of these on the pattern.

10. The method as claimed in any of claims 1 - 8, wherein the stamping procedure comprises the following steps:

preparation of a film containing the reporter molecule, target analyte or complex between the reporter and target analyte from solution on said substrate;

placing a patterned or non-patterned stamp on the film on the substrate for a period of time, the stamp being capable of modifying the surface of the substrate to exhibit said hydrophilic and hydrophobic areas;

bringing a solution containing one or more of a reporter molecule, a target analyte, a receptor molecule or a complex between these into conformal contact with the pattern;

incubating a period of time;

removing excess solution is removed from the surface.

11. The method as claimed in any of claims 9 or 10, wherein the step of removing the excess solution is carried out by blowing an inert gas, such as nitrogen on the surface.

12. The method as claimed in any of the preceding claims, wherein the stamping procedure comprises applying a layer of plastomer molecules, suitably polyolefin plastomer (POP) molecules, preferably PDMS molecules on the substrate.

13. A method of determining selected properties of analytes, comprising: detecting a change of a property of a reporter molecule, provided on a device as claimed in any of claims 1-12, in response to an interaction between the reporter and an analyte; and using the detected change to determine said selected property of said analyte.

14. The method as claimed in claim 13, wherein the change of said property is detected by measuring fluorescence, Förster resonance energy transfer (FRET), quenching of emitted light, absorption, impedance, refraction index, mass, visco-elastic properties, thickness or other physical properties.

15. A biosensor device, comprising a patterned substrate having hydrophilic and hydrophobic areas, and at least one reporter molecule, a property of which is detectable, said reporter molecule being bound to selected ones of said hydrophilic and hydrophobic areas on said patterned substrate.

16. The biosensor device as claimed in claim 15, wherein said reporter molecule is selected from the group consisting of a conjugated polyelectrolyte, copolymers or homopolymers of thiophene, pyrrole, aniline, furan, phenylene, vinylene or derivatives thereof.

17. The biosensor device as claimed in claim 16, wherein said conjugated polyelectrolyte is fluorescent.

18. The biosensor device as claimed in any of claims 15 - 17, wherein said reporter molecule is capable of interaction with a biomolecule, and wherein said interaction will cause a change in said detectable property.

19. The biosensor device as claimed in any of claims 15 – 18, wherein said substrate comprises silicon wafers, glass, glass slides, glass beads, glass wafers, silicon rubber, polystyrene, polyethylene, fluorinated hydrocarbon polymers, silica gel beads, gold, indium tin oxide-coated materials, filter paper made from nylon, cellulose or nitrocellulose, standard copy paper or variants thereof or separation media or other chromatographic media

20. The biosensor device as claimed in any of claims 15 – 19, wherein selected ones of said areas further comprise any of one or more receptor molecules and one or more target analytes alone or in combination, and forming a complex with said reporter molecule.

21. The biosensor device as claimed in any of claims 15 – 20, wherein said receptor molecules are selected from the group consisting of peptides, carbohydrates, nucleic acids, lipids, pharmaceuticals, antigens, antibodies, proteins, organic polymers or combination of these molecules capable of interacting with said target analyte.

22. The biosensor device as claimed in any of claims 20 - 21, wherein said target analytes are selected from the group consisting of cells, viruses, bacteria, spores, microorganisms, peptides, carbohydrates, nucleic acids, lipids, pharmaceuticals, antigens, antibodies, proteins, enzymes, toxins, organic polymers or combinations of these molecules that are capable of interacting with said receptors or reporter/receptor complexes.

23. A biosensor apparatus, comprising a biosensor device as claimed in claim 15, said biosensor device being located in a receptacle, suitably a flow cell, the apparatus further comprising means for detecting said detectable property.

24. A biosensor apparatus, comprising a biosensor device as claimed in claim 15, said biosensor device being located in a receptacle, suitably a flow cell, the apparatus further comprising means for detecting said detectable property.